CHARACTERIZATION OF THE EC2APEA ANTIVIRAL PROTEIN THROUGH PHAGE INFECTIONS AND TOXICITY ASSAYS

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The intense coevolutionary arms race between bacteria and phages has resulted in an unexpectedly wide and diverse array of antiphage defense systems [1]. While well-characterized antiviral systems such as CRISPR-Cas and restriction-modification directly target bacteriophage genetic material, abortive infection (Abi) systems function by activating toxic effectors upon infection, causing inhibition of metabolism or programmed death of the host cell to halt bacteriophage proliferation and protect the bacterial colony [2]. Many Abi systems rely on proteins containing HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domain, which possesses endoribonuclease activity and is important in a variety of bacterial immune responses [3].

Our research focuses on one such Abi system ApeA which encodes a single protein ApeA with a HEPN domain variant as its key functional feature. While ApeA was previously shown to defend against a limited set of classic T phages [4], the molecular mechanisms were not yet clear. Our earlier work expanded the known antiphage spectrum of ApeA homologs and identified a highly active homolog Ec2ApeA. Structural analysis of Ec2ApeA revealed a loop, prominently changing its conformation during activation. The protein was also co-purified with a dinucleotide, suggesting a potential activation mechanism.

Here we aimed to explore the activation mechanism of Ec2ApeA further by assesing the functional importance of the structurally dynamic loop, as well as the potential dinucleotide signal in the cell. Targeted mutagenesis was used to evaluate the role of the conformationally dynamic loop and co-expression of a dinucleotide cleaving enzyme was employed to test the requirement of dinucleotides as signaling molecules in Ec2ApeA activation.

These experiments demonstrated that both the conformationally dynamic loop and the dinucleotide signal are critical for Ec2ApeA protein activation.

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